Inflammasome Inhibition: Putting Out the Fire

Mihai G. Netea[1,*] and Leo A.B. Joosten[1,*]
[1]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands
*Correspondence: mihai.netea@radboudumc.nl (M.G.N.), leo.joosten@radboudumc.nl (L.A.B.J.)
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NLRP3-inflammasome activates caspase-1 and processes pro-IL-1β and pro-IL-18 into the active cytokines. Two recent studies describe specific inhibitors of NLRP3 inflammasome that inhibit IL-1β release and inflammation. The specificity and potency of these compounds gives hope that a targeted approach to inhibit NLRP3-driven inflammation may be just around the corner.

Activation of some of the most biologically active members of the IL-1 family, such as IL-1β or IL-18, is dependent on the cleavage of inactive precursors. This process is mediated by activation of protein platforms called inflammasomes that activate inflammatory caspases that in turn lead to cleavage and activation of IL-1β and IL-18 (Lamkanfi and Dixit, 2014). "Classical" (or "canonical") inflammasomes activate caspase-1, while "non-canonical" inflammasomes activate caspase-11. Inflammasomes include either receptors of the NOD-like receptor (NLR) family of proteins (e.g., NLRP3, NLRP1, and NLRC4) or AIM receptors (AIM2 inflammasome) (Lamkanfi and Dixit, 2014). Specific inhibition may give the opportunity for targeted therapy in particular diseases in which each of these inflammasome complexes are involved in pathogenesis. While endogenous inhibitors of inflammasome such as IL-37 or type I interferons are well known, very few pharmacological inhibitors that can be used in the patients have been described to date (Ahn et al., 2014; Juliana et al., 2010). Moreover, these pharmacological inhibitors are relatively non-specific and have low effectiveness. Due to these limitations, broad blockade with anti-IL-1β antibodies or recombinant IL-1 receptor antagonist represents the mainstay of anti-IL-1 therapy to date. In addition, no anti-IL-18 treatment is currently available.

An important step toward the goal of targeted anti-IL-1/IL-18 therapy was made by two studies published recently in *Nature Medicine* (Coll et al., 2015; Youm et al., 2015). Changes in the metabolic processes during specific ketogenic diet or starvation lead to the use of alternative sources of energy. In periods characterized by glucose shortage, ketone bodies have been shown to enter metabolic pathways and to be used as an alternative ATP source. In a recent study in *Nature Medicine*, Youm et al. (2015) reported that the ketone body β-hydroxybutyrate (BHB) specifically inhibits NLRP3 inflammasome activation and the production of active IL-1β and IL-18. This effect led to inhibition of these proinflammatory cytokines by several well-known NLRP3 activators both in mouse bone-marrow-derived macrophages and human monocytes. In addition, when administered in vivo in formulations containing nanolipogens aimed to improve its bioavailability, BHB inhibited NLRP3 inflammasome activation in response to monosodium ureate (MSU) crystals, the causative agent of gout. In contrast, no effect on the activation of NLRP1, NLRC4, or AIM2 inflammasome has been observed. Similarly, in mouse models mirroring the human gain-of-function mutations in NLRP3 that lead to the auto-inflammatory syndromes Muckle-Wells (MWS) and familial cold auto-inflammatory syndrome (FCAS), BHB induced by a ketogenic diet potently reduced IL-1β secretion and peritoneal inflammation (Youm et al., 2015).

The study of Youm and colleagues is a novel argument for the strong interaction between metabolic processes and immune stimulation. Increasing amounts of evidence point toward a crucial role for cellular metabolism of glucose during the monocyte/macrophage, dendritic cell, and lymphocyte activation, with oxidative phosphorylation being the main pathway being used by naive or tolerant cells, while cellular metabolism shifts toward aerobic glycolysis during activation—the so-called Warburg effect (Tannahill et al., 2013). In addition, a recent report showed that fatty acid synthesis is a strong inducer of NLRP3 activation, suggesting that this may represent another target for anti-inflammatory therapy (Moon et al., 2015). Interestingly, the metabolites acetoacetate or butyrate, which are structurally related to BHB, did not inhibit activation of the inflammasome, although they have been previously shown to have important anti-inflammatory properties (Vinolo et al., 2011).

A different approach was initiated by Coll et al. (2015) who report identification of a synthetic NLRP3 inhibitor of the inflammasome that they termed MCC950. Interestingly, this compound has been previously described as an inhibitor of IL-1β, but its effects on the inflammasome activation were not known. MCC950 blocked NLRP3 activation in both in vitro mouse macrophage stimulation as well as in vivo experimental models such as endotoxemia and a mouse model of multiple sclerosis. Similar to Youm and colleagues, Coll et al. (2015) also showed MCC950 to ameliorate the symptoms of MWS in a mouse model. Even more importantly, blockade of NLRP3 activation by MCC950 led to inhibition of IL-1β production by leukocytes isolated from patients with MWS.

The mechanisms through which BHB and MCC950 inhibit NLRP3 activation differ. BHB inhibits the K^+ efflux and ASC oligomerization, while MCC950 does not. Interestingly, and in contrast to BHB, which only inhibited activated caspase-1, MCC950 inhibits both canonical caspase-1 activation and the non-canonical caspase-11 pathway of IL-1β activation and pyroptosis induction (Coll et al., 2015) (Figure 1). This differential effect opens the possibility to specifically approach the treatment of conditions in which either capsase-1 and/or caspase-11 are involved.

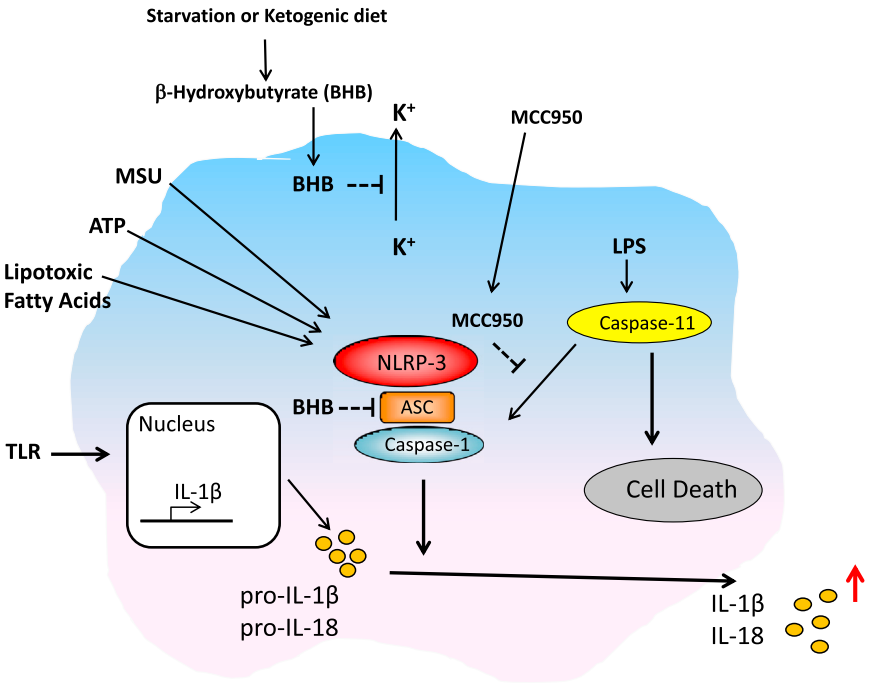

Figure 1. Schematic Illustration of the Molecular Mechanisms of NLRP3 Activation and the Targets of β-hydroxybutyrate and the Pharmacologic Inhibitor MCC950

NLRP3 inflammasome is activated by MSU, ATP, or lipotoxic fatty acids. Caspase-1 processes intracellular pro-IL-1β and pro-IL-18 into the active cytokines. K^+ efflux is needed for optimal inflammasome activation. BHB inhibits the efflux of K^+, while both BHB and MCC950 inhibit ASC oligomerization and speck formation. Interestingly, MCC950 inhibits both caspase-1 and caspase-11, while BHB only affects caspase-1 activation.

While the two studies are very important steps toward the aim of specific inflammasome-dependent therapy, important challenges remain. While the specific effect of NLRP3 inhibition gives hope that IL-1 inhibition will be selective and probably not complete, thus sparing the activation of other inflammasomes (e.g., NLRC4 and AIM2 inflammasomes) involved in host defense, this should be formally tested in infection models. Moreover, one should also test the possible effects of these inhibitors in infection models in which NLRP3 inflammasome does play a role, such as disseminated candidiasis (Gross et al., 2009). In addition, one should consider the most likely inflammatory diseases in which NLRP3 inflammasome plays a role and in which these compounds are likely to be effective, especially taking into account the known inflammasome-independent processing of pro-IL-1β by neutrophil-derived serine proteases. Thus, while monocyte/macrophage-dependent inflammatory processes are likely to react to the novel NLRP3 inhibitors, this is less likely to happen in models of inflammation in which neutrophils play a major role. One such condition is gout (Joosten et al., 2010), and testing the effectiveness of these compounds in experimental models of gout mimicking human disease by intra-articular injection of uric acid crystals is warranted.

To conclude, the two important studies by Youm et al. (2015) and Coll et al. (2015) open a new chapter in our anti-inflammatory therapeutic armamentarium in which NLRP3-specific inhibition should have an important place.

REFERENCES

Ahn, H., Kim, J., Jeung, E.B., and Lee, G.S. (2014). Immunobiology *219*, 315–322.

Coll, R.C., Robertson, A.A., Chae, J.J., Higgins, S.C., Muñoz-Planillo, R., Inserra, M.C., Vetter, I., Dungan, L.S., Monks, B.G., Stutz, A., et al. (2015). Nat. Med. *21*, 248–255.

Gross, O., Poeck, H., Bscheider, M., Dostert, C., Hannesschläger, N., Endres, S., Hartmann, G., Tardivel, A., Schweighoffer, E., Tybulewicz, V., et al. (2009). Nature *459*, 433–436.

Joosten, L.A., Netea, M.G., Mylona, E., Koenders, M.I., Malireddi, R.K., Oosting, M., Stienstra, R., van de Veerdonk, F.L., Stalenhoef, A.F., Giamarellos-Bourboulis, E.J., et al. (2010). Arthritis Rheum. *62*, 3237–3248.

Juliana, C., Fernandes-Alnemri, T., Wu, J., Datta, P., Solorzano, L., Yu, J.W., Meng, R., Quong, A.A., Latz, E., Scott, C.P., and Alnemri, E.S. (2010). J. Biol. Chem. *285*, 9792–9802.

Lamkanfi, M., and Dixit, V.M. (2014). Cell *157*, 1013–1022.

Moon, J.S., Lee, S., Park, M.A., Siempos, I.I., Haslip, M., Lee, P.J., Yun, M., Kim, C.K., Howrylak, J., Ryter, S.W., et al. (2015). J. Clin. Invest. *125*, 665–680.

Tannahill, G.M., Curtis, A.M., Adamik, J., Palsson-McDermott, E.M., McGettrick, A.F., Goel, G., Frezza, C., Bernard, N.J., Kelly, B., Foley, N.H., et al. (2013). Nature *496*, 238–242.

Vinolo, M.A., Rodrigues, H.G., Nachbar, R.T., and Curi, R. (2011). Nutrients *3*, 858–876.

Youm, Y.H., Nguyen, K.Y., Grant, R.W., Goldberg, E.L., Bodogai, M., Kim, D., D'Agostino, D., Planavsky, N., Lupfer, C., Kanneganti, T.D., et al. (2015). Nat. Med. *21*, 263–269.